THE HARRISON LECTURE

The history and role of *Mycoplasma genitalium* in sexually transmitted diseases

David Taylor-Robinson

It is an honour to be invited by the officers of the Medical Society for the Study of Venereal Diseases (MSSVD) to give the eighth Harrison Lecture and, indeed, to follow in the footsteps of those who have been leaders and influential in the field. Lawrence Whittaker Harrison was such a man.[1] He was born just outside Blackburn, Lancashire, in 1876 and, clearly, he was a Blackburn Rover, moving away to be educated at Manchester Grammar School and then to Glasgow University to study medicine. Two years after qualification, Harrison joined the Army Medical Services and served in the South African war and then in India where his interest in venereal diseases was first aroused. In the First World War, working in France, he saw and managed many cases of venereal disease, all of which was to influence the path he took subsequently. Harrison was a great believer in laboratory experience as a guide to clinical judgement. This stemmed from the fact that he had such experience early in his career, working from 1910 to 1914 on the Wasserman reaction, such that the modification he made to this (the Harrison-Wyler method) was used for a considerable number of years thereafter. After the war he was offered a post in Edinburgh to lecture on venereal diseases and to set up a clinic there but, instead, became Advisor to the Ministry of Health in London, a position that also involved setting up a venereal disease clinic at St Thomas's Hospital. The practice of medicine and the principles of management that Harrison laid down in instituting that clinic so many years ago are those behind the running of genitourinary medicine (GUM) clinics today. He was a founder member and twice President of the MSSVD and was widely influential.

Associations with Liverpool

I was born not very far from Blackburn, in fact in Bolton, and I became a Bolton Wanderer. However, my association with Liverpool is strong. I wandered with my parents to Liverpool where I went to the Liverpool Collegiate School and, later, to Liverpool University Medical School. My father was, what was called in those days, the City Bacteriologist and Associate Professor in the University. So the opportunity for looking down a microscope came to me at an early age and possibly had some influence on my future career. My youngest brother, John, is a general practitioner in Liverpool. My younger brother Carl, who was Senior Lecturer in the Department of Medical Microbiology, sadly died a few years ago. He was a virologist and mycoplasmas to him were not the same as they are to me because they can be a considerable nuisance to virologists as contaminants of cell cultures.[2]

Characteristics of mycoplasmas

The ability to contaminate cell cultures is just one of the characteristics of mycoplasmas (table 1). It seems that they have devolved from bacteria by gene deletion so that they no longer have the genetic capability to make a rigid cell wall. The size of the smallest viable forms is about that of the pox viruses. Indeed, mycoplasmas are small enough to penetrate the surface of agar medium and as a consequence of growth in the depth of the agar[3] (fig 1), the classical "fried-egg"-type colonies are seen when viewed microscopically from above (fig 2a). If looked at obliquely from beneath with adequate lighting the colonies appear as "flying saucers" (fig 2b).

Features of M pneumoniae

As a result of working on respiratory viruses in my early career at the Common Cold Unit under the direction of David Tyrrell, I had two sabbatical periods in the early 1960s at the National Institutes of Health (NIH) in Bethesda, Washington, USA. On both occasions I worked in the Laboratory of Infectious Diseases headed by Robert Chanock. I was fortunate the first time in arriving just after the Eaton agent, later called *Mycoplasma pneumoniae*,[4] had been grown on acellular medium.[5] Working on this micro-organism and noting its haemolytic activity when colonies on agar were overlaid with guinea pig erythrocytes[6] was my introduction to mycoplasmology. Considering other characteristics of *M pneumoniae* at this stage is not

Table 1 Characteristics of mycoplasmas

Grow in cell-free medium
Usually produce "fried-egg" type colonies
Smallest viable forms about 200–300 nm
Often spherical but some, such as *M pneumoniae* and *M genitalium*, have a bottle- or pear-shaped morphology
Cell wall absent and bounded by a triple-layered membrane
Contain DNA and RNA
Growth inhibited by broad-spectrum antibiotics; however, resistant to penicillins (due to lack of cell wall) and rifampins, and resistance to others may develop
Growth inhibited by antibody, particularly in the presence of complement
Tend to be host-specific
Some immunostimulate; others immunosuppress
Cause respiratory and genital tract disease and, in animals, mastitis
Contaminate cell cultures

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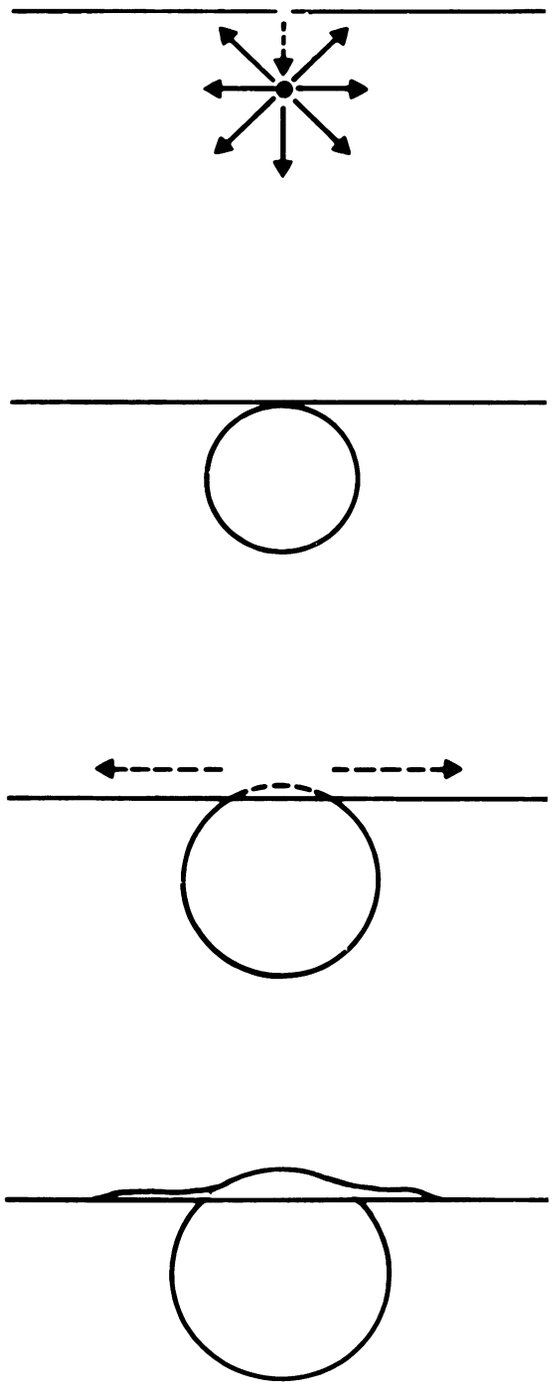

Figure 1 Schematic representation of a mycoplasma colony developing in, and on, agar medium.

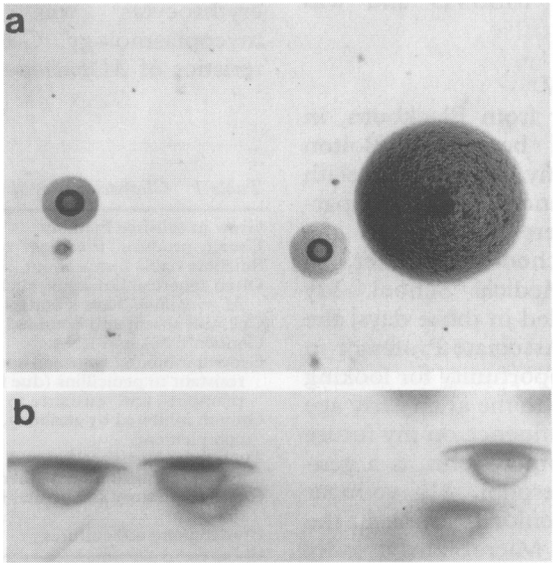

Figure 2(a) Typical "fried-egg" mycoplasma colonies close to a larger bacterial colony, viewed from above by transmitted light. (b) Mycoplasma colony viewed obliquely from below. Growth in the agar gives the appearance of a "flying saucer".

irrelevant because there are important analogies to be drawn between this mycoplasma and *Mycoplasma genitalium*. The Biberfelds in Sweden[7] were the first to note that *M pneumoniae* has a bottle- or pear-shaped morphology, the terminal nose-like structure being used for attachment to epithelial cells. This is mediated by the 170 kDa P1 protein which is part of the outer membrane.[8] The application of a monoclonal antibody, prepared against the P1 protein and tagged with ferritin, to this mycoplasma, results in its attachment mainly to the terminal structure.[9] In other words, the P1 protein is the adhesin which enables the mycoplasma to attach to cells. The phenomenon of haemadsorption illustrates the adhesion process and the attachment of the organisms to respiratory epithelial cells following experimental infection of the hamster lung is shown in fig 3. The damage caused by *M pneumoniae* is to a large extent immunologically mediated, being a secondary cellular over-response of the immune system to a primary infection.[10] Finally, it should be pointed out that *M pneumoniae* organisms may be difficult to eradicate from the respiratory tract, despite the fact that they may be sensitive to the antibiotic in vitro.[11]

Work on urethritis leading to detection of M genitalium

During my second sabbatical period at the NIH I spent some time working together with Robert Purcell on mycoplasma organisms that, in those days, were called T-strains and which were later ascribed to the genus and species, *Ureaplasma urealyticum*.[12] I returned from the United States with an understanding of how to isolate ureaplasmas, as these organisms became known trivially, and began to collaborate with Eric Dunlop at the London Hospital who was working on non-gonococcal urethritis (NGU). Together we examined the role of ureaplasmas in post-gonococcal urethritis.[13] This was my introduction to studying urethritis in men and it was followed by a series of studies on NGU in which Mary Prentice,[14] Emile Coufalik,[15] Pat Munday,[16] Nashat Hanna,[17] David Hawkins,[18] Phillip Hay,[19] and Patrick Horner,[20] together with Brenda Thomas, Pat Furr, Eli Fontaine and others, have made invaluable contributions.

During the course of these investigations, it became apparent to me that it was unlikely that NGU in all patients could be accounted for by infection with *Chlamydia trachomatis* or *U urealyticum*. I had looked at wet preparations of urethral discharge microscopically and wondered whether motile spiral forms that I saw might be spiroplasmas. These are motile, helical mycoplasmas found in plants and insects.[21] My thought was preconditioned because I had knowledge of these organisms, having made some contribution[22] to the serological characterisation of the first of these to be isolated, namely *Spiroplasma citri*, by Josy Bové and colleagues in Bordeaux, France.[23] It occurred to me that there might be a human counterpart. Subsequent to the first isolation of spiroplasmas, Joseph Tully and colleagues

Table 2 Primary sites of colonisation, metabolism and pathogenicity of mycoplasmas of human origin

Species	First report of isolation	Primary site of colonisation		Metabolism of		Pathogenicity
		Oropharynx	Genirourinary tract	Glucose	Arginine	
M hominis	1937	+	+	−	+	+
M fermentans	1952	+	+	+	+	?
M salivarium	1953	+	−	−	+	−
U urealyticum†	1954	+	+	−	−	+
M primatum	1955	−	+	−	+	−
M pneumoniae	1962	+	−	+	−	+
A laidlawii	1964	+	−	+	−	−
M orale	1964	+	−	−	+	−
M buccale	1965	+	−	−	+	−
*M pirum**	1968	?	?	+	+	−
M faucium	1969	+	−	−	+	−
M lipophilum	1974	+	−	−	+	−
M genitalium	1981	? +	+	+	−	+
A oculi	1987	?	−	+	−	−
M spermatophilum	1991	−	+	−	+	−
M penetrans	1991	−	+	+	+	?

* human origin debatable
† metabolizes urea

at the NIH, Bethesda, had developed SP4 medium which was invaluable not only for the isolation of spiroplasmas but also for the isolation of other mycoplasmas, such as *M pneumoniae*,[24] which were difficult to recover. Thus, as part of a collaborative venture, I took urethral specimens from 13 men with NGU to Tully's laboratory. Inoculation of the SP4 medium with these specimens was followed about a month later, just as I was about to return to the UK, by an acidic colour change in the medium containing two of the specimens; this could have occurred for various reasons but the change was subculturable and electron microscopic examination revealed a micro-organism that certainly was not a spiroplasma, but which had a morphology very much like that of *M pneumoniae*[25]; in other words, pear-shaped with a terminal structure which on sectioning was seen to contain a central dense core (fig 4). Further investigations revealed that this mycoplasma was serologically different from all other known mycoplasmas and eventually it was termed *M genitalium*,[26] being the twelfth mycoplasmal species known to exist in and be recovered

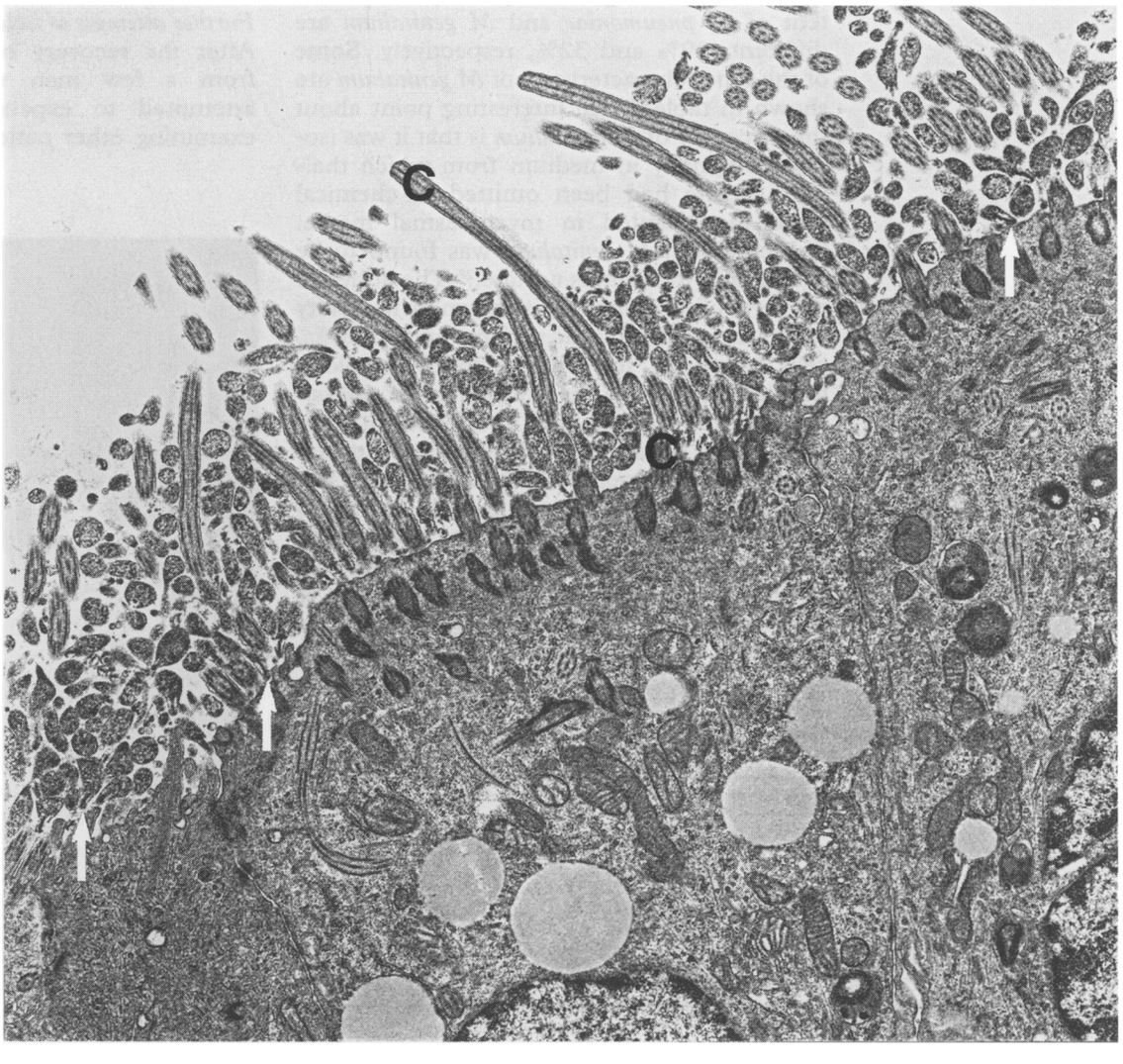

Figure 3 Electron micrograph of ciliated epithelial cells in the tracheal mucosa of a hamster infected with M. pneumoniae. *Note cilia(c) and individual organisms (arrowed), some with the specialised terminal structure oriented towards the membrane of the host cell (× 13,000).*

Table 3 Characteristics of M genitalium

Genome size: 600 kbp
Guanine + cytosine content: 32%
Metabolises glucose, but not arginine or urea
Grows very slowly, but more rapidly on repeated subculture
Produces some "fried egg"-like colonies in atmosphere of N_2–5% CO_2 but not in air–5% CO_2
Predominantly bottle-shaped with a terminal rod-like structure
Growth inhibited by thallous acetate
Growth inhibited by tetracyclines, erythromycin and other antibiotics
Adheres to glass and plastic surfaces
Exhibits haemadsorption : adhesion (MgPa) is protein of 140 kDa
Exhibits gliding motility

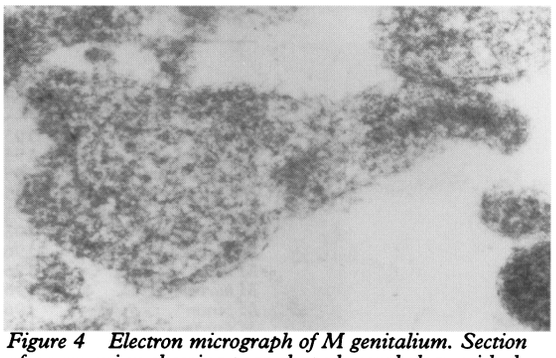

Figure 4 Electron micrograph of M genitalium. Section of an organism showing pear-shaped morphology with the terminal structure containing a central dense core (× 100 000).

from humans (table 2). I noted with interest and amusement some years later that the appearance of squash in a vegetable market in the United States had an uncanny resemblance to the morphology of *M genitalium* (fig 5).

Features of M genitalium

Despite the fact that *M pneumoniae* and *M genitalium* are structurally and to some extent antigenically related (*vide infra*), they are not, of course, genomically the same. *M genitalium* has the smallest genome size of all the mycoplasmas (600 kbp)[27]; that of *M pneumoniae* is larger (800 kbp) and for comparison the genome sizes of *C trachomatis* and *Escherichia coli* are 1450 kbp and 4700 kbp, respectively. The guanine plus cytosine content of *M pneumoniae* and *M genitalium* are different, 39% and 32%, respectively. Some of the other characteristics of *M genitalium* are shown in table 3. An interesting point about the discovery of *M genitalium* is that it was isolated originally in medium from which thallous acetate had been omitted, a chemical often incorporated in mycoplasmal media. Subsequently, *M genitalium* was found to be susceptible to thallous acetate.[28] The adhesive property of *M genitalium* is shown by its ability to attach to glass, plastic surfaces and epithelial cells,[26] and by the phenomenon of colony haemadsorption.[26] The adhesin of *M genitalium* (MgPa) is 140 kDa.[29] It is different from that of *M pneumoniae* (170 kDa), but has some shared properties.[30] By the use of a ferritin-labelled monoclonal antibody to the *M genitalium* adhesin protein, it has been possible to show that it clusters on the terminal portion of the organism.[29] Furthermore, it is an immunodominant protein.[30] Mutants of *M genitalium*, which do not produce the 140 kDa protein, do not exhibit colony haemadsorption.[31] This is a further illustration of the way in which the protein is involved in adherence, an important first step in the pathogenicity of this mycoplasma. Recently, it has been demonstrated that *M genitalium* not only adsorbs to epithelial cells but also invades them and becomes intracellular.[31 32] Finally, despite gene deletion, *M genitalium* still possesses sufficient genomic make-up to be actively motile.[33] Because there is probably insufficient genomic material to cater for frivolous activities, the assumption is that motility is important perhaps as a means of penetrating the mucous layer covering mucosal epithelial cells and enabling the mycoplasma to attach to and invade the cells.

Further attempts to isolate M genitalium

After the recovery of *M genitalium* initially from a few men with acute NGU, we attempted to expand this observation by examining other patients with NGU, as well

Table 4 Detection of M. genitalium in men with and without non-gonococcal urethritis (NGU)

Study reference	No (%) patients-positive/no treated with		
	NGU*	Gonorrhoea	No disease
25	2 (15%)/13	—	—
17	7 (32%)/22	2 (12%)/17	2 (10%)/20
36	7 (12%)/61	3 (14%)/21	10 (12%)/84
19	9 (50%)/18	0 (0%)/9	1 (14%)/7
19	13 (19%)/68	—	—
20	24 (23%)/103	—	3 (6%)/53
39	13 (27%)/48	0 (0%)/4	4 (8·5%)/47

* Chlamydia-positive and chlamydia-negative

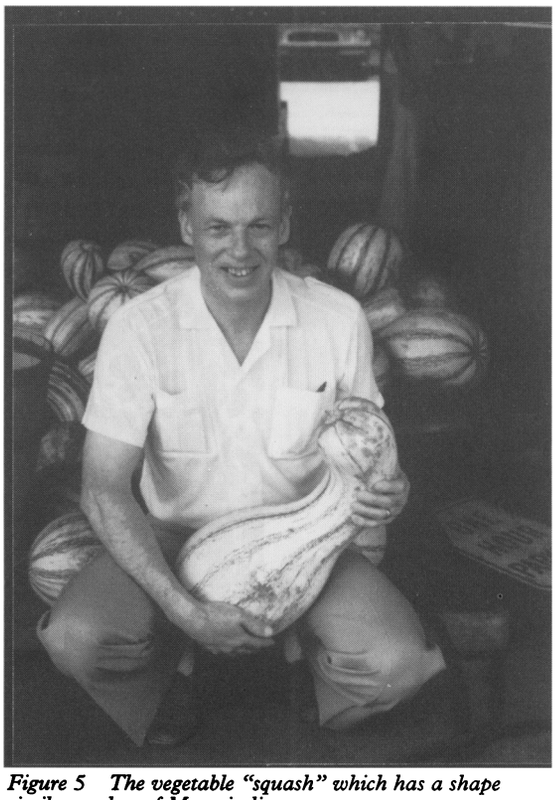

Figure 5 The vegetable "squash" which has a shape similar to that of M genitalium

as men with gonorrhoea and those who had no urethritis when they were seen at the GUM clinic. Subculturable colour changes occurred in medium inoculated with specimens from seven of 22 patients with NGU, two of 17 with gonorrhoea and two of 20 without urethritis[17] (table 4). There were, however, two problems with this study. First, the colour changes could be regarded only as presumptive of the existence of *M genitalium* because specific identification was hindered by many of the changes not being reproducible after the media had been stored frozen; and second, the changes had taken one to almost five months to occur on incubation of the media at 37°C. Clearly, it is not feasible to undertake sensible experimentation under such circumstances and, therefore, efforts were made to improve the growth medium for *M genitalium*. It was possible to show, using a laboratory-passed strain of *M genitalium*, that, for example, fetal calf serum was superior to horse serum when incorporated in Edward-type medium, and that the addition of glutamine to SP4 medium that had been stored at 4°C improved its performance considerably (Furr PM, Taylor-Robinson D, unpublished data). However, these and other attempted improvements did not culminate in a medium which was any more sensitive for the primary isolation of *M genitalium*.

As a result of the difficulty of culturing *M genitalium*, several workers invested in efforts to develop DNA probes for the mycoplasma. One of these probes[35] was used by Hooton and colleagues[36] to examine genital specimens from men ascribed to a number of clinical categories. They could not demonstrate that the prevalence of *M genitalium* in men with acute chlamydia-positive or chlamydia-negative NGU was significantly different from that in men who had gonococcal urethritis or no urethritis (table 4). They did show, however, that the mycoplasma was present more frequently in homosexual men than in heterosexual men and, furthermore, that there was a significantly increased prevalence in individuals who had persistent or recurrent NGU. We and other investigators took a different tack and developed the polymerase chain reaction (PCR)[37 38] which was coming into vogue about this time as a very sensitive means of detecting micro-organisms. Thus, my colleagues Helen Palmer and Claire Gilroy were able to demonstrate that the PCR could detect as little as 10^{-15} grams of *M genitalium* DNA.[37] It was then possible, with this technique in our armamentarium, to begin to look at the relationship of *M genitalium* with, for example, acute and chronic NGU, chronic prostatitis, pelvic inflammatory disease (PID) and arthritis.

Association of M genitalium with disease

Acute non-gonococcal urethritis. The availability of the PCR provided the opportunity to re-evaluate our earlier study[17] of men with and without urethritis. After a number of years, not all specimens were available for testing; however, we found *M genitalium* in nine of 18 specimens from men with NGU, in none of nine specimens from men with gonorrhoea and in one of seven controls without urethritis[19] (table 4). This provided some assurance that the colour changes we had seen originally were due to *M genitalium* and could be regarded as more than presumptive. Subsequently, we detected *M genitalium* DNA in 19% of another group of men who had acute NGU[19] and most recently in 23% of symptomatic men with NGU but in only 6% of asymptomatic subjects[20] (table 4), a difference which was statistically significant. Furthermore, the occurrence of *M genitalium* was independent of the occurrence of *C trachomatis*. These findings were similar to those reported recently by Jensen and colleagues in Denmark[39]; they detected *M genitalium* in 25% of men with urethritis, which was significantly greater than the 8·5% of those without disease (table 4).

Attempts to look for antibody responses to *M genitalium* in men with NGU have been hampered by the fact that there is strong serological cross-reactivity between *M genitalium* and *M pneumoniae*. This is exhibited particularly in the complement-fixation test[40] and possibly least by microimmunofluorescence.[41] We developed the latter procedure for *M genitalium*[42] and then tested sera that had been collected in the earlier study of NGU.[17] Some four-fold rises and four-fold falls in antibody titre were detected, but further sera need to be examined and, if possible, with a more discriminating test. The existence of cross-reacting antibody raises the question of whether infections by *M pneumoniae* in the respiratory tract could influence *M genitalium* in the urogenital tract, either by protecting against disease or by enhancing it, in the same way that consideration might be given to the possibility that *C pneumoniae* in the respiratory tract could have an effect on *C trachomatis* in the urogenital tract. No pertinent observations have been made in either context, but they seem worthy of investigation.

Antibiotics which differentiate between micro-organisms on the basis of their sensitivity have sometimes been useful in helping to define aetiological associations,[15] but antibiotics that affect *M genitalium* alone or that have no effect on this mycoplasma but have on other putative pathogens are unlikely to exist. Hence, this approach to defining the role of *M genitalium* in NGU would appear thwarted. In contrast, the results of inoculating non-human male primates have given support to the idea that *M genitalium* is important in NGU.[43–45] The organisms did not cause disease following intra-urethral inoculation of male rhesus monkeys and have caused urethritis in only a small proportion of macaque monkeys. However, of 10 male chimpanzees inoculated intraurethrally, eight developed an urethral polymorphonuclear leucocyte response, the organisms being recovered from seven of them, either intermittently or persistently, in some cases for more than 18 weeks. Eight of the animals developed four-fold or

greater rises in antibody titre which usually did not occur until four or five weeks after inoculation; this cautions against acquiring a "convalescent"-phase serum too early in the human situation. In two cases, the organisms were also recovered from the blood,[44] indicating the invasiveness of this micro-organism.

Chronic non-gonococcal urethritis and chronic abacterial prostatitis. In the case of chronic NGU, that is persistent or recurrent disease following an acute attack, Hooton and colleagues[36] noted an association with *M genitalium*, as mentioned previously. We have detected the mycoplasma by the PCR technique in the urethra of about 20% of men when the disease is in the chronic phase.[19] Whether *M genitalium* has been carried since the original attack of NGU is a moot point since men presenting with chronic disease have almost always received one or two courses of tetracyclines previously and sometimes a course of erythromycin. However, it is pertinent to reflect that *M pneumoniae* organisms are not easily eliminated from the respiratory tract,[11] despite the fact that individuals are given adequate doses of antibiotics to which the organisms are sensitive in vitro. Very few of the men with chronic disease have had evidence of a persisting chlamydial infection and the detection of *M genitalium* has coincided with the objective presence of a purulent urethral discharge and in a few cases it has not been detected when the discharge has resolved following a six-week course of erythromycin.[19] Nevertheless, the role of *M genitalium* in chronicity remains unclear.

Somewhat surprisingly, *M genitalium* has not been detected in transperineal derived prostatic biopsy specimens taken under ultrasound control from patients with chronic abacterial prostatitis (Gilroy CB, Doble A, Taylor-Robinson D, unpublished data) and clearly there is no evidence for its involvement. Of course, there is, as yet, no certainty that *M genitalium* causes chronic NGU, but assuming that it does, a feasible mechanism might involve heat-shock proteins. These have been found in the mycoplasmal species studied so far.[46] Although such proteins have not been sought specifically in *M genitalium*, antibody to the common antigen of *Legionella spp* cross-reacts with a 62 kDa protein of *M genitalium*,[47] so that there is some indirect evidence for their presence. The common antigen may be a specific target for the $\gamma\delta$-subset of T-cells and trigger a host autoimmune response. An immunological overresponse to *M pneumoniae* as a cause of pneumonia has analogous overtones.

Although *M genitalium* has been found in the respiratory tract,[48] it seems from the current evidence that its preferred mucosal site is the urogenital tract. However, a possible preference for the intestinal tract needs to be considered, particularly in view of the fact that *M alvi* and *M sualvi*, which have the same structural configuration as *M genitalium*, have been found in both the genital and intestinal tracts of cattle[49] and swine,[50] respectively. For this reason, it would seem worthwhile seeking *M genitalium* in the human intestinal tract and defining whether it has a role in homosexual, as well heterosexual, NGU.

Pelvic inflammatory disease. Turning to the possible role of *M genitalium* in diseases of women, the mycoplasma has been detected by the PCR technique in the lower genital tract of about one-fifth of women attending the GUM clinic at St Mary's Hospital[51] and in cervical samples from five of 74 women in Copenhagen.[38] This raises the question of whether it might be involved in causing disease in the upper tract. In various monkey models, the mycoplasma has been shown to cause lower genital-tract inflammation, and salpingitis in marmosets, grivet monkeys and baboons.[44 45] Other support for a role of *M genitalium* in PID comes from the fact that it adheres to human fallopian tube epithelial cells in organ culture,[52] and a four-fold or greater rise in the titre of antibody to *M genitalium*, measured by microimmunofluorescence, was detected in about one-third of women with acute PID who did not have evidence of gonococcal, chlamydial or *M hominis* infection.[53] However, not all have found this to be the case[54] and resolution of the role of *M genitalium* in PID will only come when specimens from the upper genital tract are examined by the PCR technique.

Arthritis. In view of the invasiveness of *M genitalium* in chimpanzees, mentioned earlier,[44] it is interesting to speculate that this mycoplasma could disseminate from the human genital tract to distant sites, for example the joints. In this regard, *M genitalium* and *M pneumoniae* have been isolated together from synovial fluid from the joint of a patient with polyarthritis.[55] This occurred after a bout of pneumonia that was considered to be due to the latter mycoplasma.[56] Most recently, in collaboration with Shula and Jacob Horowitz in Israel, *M genitalium* has been detected by the PCR technique in synovial fluid from the joints of two individuals[57]; one had sexually acquired reactive arthritis and the other had sero-negative rheumatoid arthritis. Quite clearly, these observations need to be expanded.

Infertility. Finally, I wish to consider the possibility that *M genitalium* might have a role in infertility, particularly male infertility. Ureaplasmas, based on their known ability to adhere to sperm, were put forward in the mid-1970s as having such a role,[58] but the matter has never been settled.[59] *M genitalium* organisms have a strong cell-adsorptive capacity, as mentioned before, and the possibility that they might adhere to the head and midpiece of sperm and alter their motility should not be ignored.

In summary, there is some compelling evidence for *M genitalium* being one of the causes of acute NGU, some evidence for its role in chronic NGU, but no evidence that it causes chronic prostatitis. Evidence that *M genitalium* has a part to play in PID needs to be strengthened and the significance of its existence in joints and the type of arthritis it is most associated with require full exploration.

A role in infertility is at the moment fanciful but, nevertheless, not impossible.

Acknowledgements

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